[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF SASKATCHEWAN]

ACTION OF ANILINE ON GLUCOSE IN ACETIC ACID SOLUTION. I

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In the past considerable work[1] has been done on the action of bases on sugars under various conditions, the effect, briefly, being one of preliminary enolization followed by dissociation of the sugar molecule. The action of acids on the hexoses,[2] leading to the production of hydroxymethyl-furfural, levulinic acid, formic acid and humus, has been investigated. Further, it has been found that amino acids act on carbohydrates[3] with the loss of carbon dioxide and formation of dark brown condensation products. However, the simultaneous effect of a weak base (aniline) and a weak acid (acetic) on glucose has not been recorded.

The more obvious effect produced when primary aromatic amines, such as aniline, and acetic acid are added to alcoholic solutions of glucose is the final appearance of a brown coloration, even at room temperature. This coloration is more rapid in such acid solutions than with glucose and aniline alone, glucose and acetic acid, aniline and acetic acid or aniline alone in alcohol. When water is used as the solvent, the colored material precipitates. It is believed that the initial step is the formation of a compound between the amine and the glucose and that this reaction is catalyzed by the acid. That the compound, glucose-anilide, is produced in acid solutions of glucose and aniline and that this reaction is more rapid in the presence of acid is shown below. That the coloration is due primarily to the formation of the compound, glucose-anilide, is indicated by the fact that this substance in acid solution gives apparently the same colored material.

The further changes, it is hoped, will be dealt with later although it might be mentioned that the color seems to be due in part to the oxidation of the aniline, but mainly to the formation of condensation products of the amine and a decomposition product of the glucose.

Experimental Part

The following experiment gives an idea of the production of color in solutions of glucose and aniline containing acetic acid as compared with comparable blanks.

Fifty cc. of an ethyl alcohol solution of 1.5 g. of glucose, 0.775 g. (1 molecular proportion) of freshly distilled aniline and 0.5 g. (1 molecular proportion) of acetic acid

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[1] Nef, *Ann.*, **403**, 204 (1914).

[2] Harrison, THIS JOURNAL, **36**, 586 (1914).

[3] Maillard, *Compt. rend.*, **154**, 66 (1912).

at room temperature (24–25°) became light yellow in two days, orange in 12, red in 18 and brown in 34. A comparable solution of glucose and aniline was a light yellow in 32 days and brown in 112. Similar solutions of aniline and acetic acid, and of aniline alone showed only a slight tinge of yellow in 120 days; at this time (120 days) a solution of glucose and acetic acid was still colorless.

In the acid solutions of glucose and aniline, the color remained brown, but after 90 days the solution failed to reduce Fehling's solution, showing that both glucose and glucose-anilide were not present as such. The alcoholic solution was then diluted to 400 cc. with water and the colored material, which separated, was filtered off and washed with water. After drying in a vacuum, it weighed 0.35 g. and had a very indefinite melting point (shrinking at 85°, black and glassy at about 116°, decomposing at 141°).

That glucose may react with aniline to form glucose-anilide[4] was recognized early in carbohydrate chemistry. Later, Irvine and Gilmour[5] proved that glucose-anilide has the butylene oxide structure and that it exists in two stereoisomeric forms, the α- and β-isomers. They further showed that the anilide is formed not only by heating glucose and aniline together in alcoholic solution, but that condensation takes place slowly when an ethyl alcohol solution of glucose is mixed with an excess of aniline and kept in the cold. A polarimetric examination of the process was thus possible. It was found that the initial dextrorotation of the solution at first increases steadily and then diminishes gradually, finally becoming constant when a decided levorotation is recorded. The initial rise, according to these authors, is due to the formation of the alpha isomer of dextrorotation; the subsequent fall is caused by the appearance of the beta isomer, the final product being essentially the *levo* isomer only. Further, they prove that the α-glucose-anilide is changed at once to the β-, on addition of a trace of acid. Finally, they point out that the anilide is readily hydrolyzed and that a methyl alcohol solution when heated with methyl iodide and silver oxide is vigorously oxidized.

Then, if the *dextro* isomer is first formed and gradually converted into the *levo* isomer, and if acid changes the former at once to the latter, it was thought that when aniline is allowed to react with glucose in the presence of an acid, the formation of the beta isomer should be hastened. It is found on following the reactions by means of the polariscope that this is true.

Thus two alcoholic solutions of 1.5 g. of glucose were made up and allowed to stand for 24 hours, so that the rotation might become constant. To one of these was added 0.5 g. (1 molecular proportion) of glacial acetic acid and to both 0.775 g. (1 molecular proportion) of freshly distilled aniline. The solutions were then made up to 50 cc. with ethyl alcohol, thoroughly mixed, and filtered into 1-dcm. polariscope tubes. Readings of the rotation were made as recorded in Table I, only the more pertinent observations being recorded. Time was computed from the addition of the aniline in all cases. After 12 days, readings on the acid solution were discontinued because of the color developed.

When glucose reacts with aniline alone, it is found that the initial dextro-

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[4] Schiff, *Ber.*, **4**, 908 (1871).

[5] Irvine and Gilmour, *J. Chem. Soc.*, **93**, 1429 (1908).

TABLE I

EFFECT OF ACETIC ACID ON THE REACTION BETWEEN GLUCOSE AND ANILINE

Glucose, 1.5 g. Aniline, 0.775 g. Solvent, 95% ethyl alcohol to a volume of 50 cc. Tube, 1 dcm. Temp., 20°.

Time	Acetic acid 0.5 g. α	No acid α
5 min.	+1.73°	+1.74°
1 hour	+1.10	+1.76
4 hours	−0.40	+1.77
6 hours	−0.72	+0.74
1 day	−1.22	+1.57
2 days	−1.25	+1.22
6 days	−1.21	−0.07
12 days	−1.15	−0.88
24 days	?	−1.26
28 days	?	−1.28
36 days	?	−1.23

rotation increases very slightly but there certainly is a distinct lag, as in six hours the rotation was identical with the initial reading. This agrees with the findings of Irvine and Gilmour[5] and is explained by these authors as due to the initial formation of the *dextro* isomer. In the presence of acid, this preliminary formation of the alpha isomer is not apparent; the rotation rapidly falls to its maximum value of —1.25° in two days. As the maximum value of —1.28° was observed in 28 days with glucose and aniline alone, the effect of acetic acid on the formation of the *l*-anilide is apparent providing, of course, that glucose-anilide is formed in the acid solutions.

That glucose-anilide is formed in acid solutions of glucose and aniline is shown by precipitating the anilide from such solutions by means of a mixture of ether and benzene. From other experiments, it was decided that it was best to use an excess of the reactants (3 molecular proportions) as with molecular quantities of aniline and acetic acid the formation of the anilide is far from complete.

Thus to a 3% alcoholic solution of glucose, which had stood for 24 hours, were added 11.62 g. of aniline (3 molecular proportions) and 7.5 g. of acetic acid (3 molecular preportions) and the volume was made up to 250 cc. with alcohol. The solution was allowed to stand at room temperature (23°) for 20 hours, when the rotation had fallen to its lowest negative value (−1.93°). The yellow solution was then cooled in an ice-salt mixture and a large excess of anhydrous ether and benzene (3:1) added. The precipitate that formed was removed by filtration, washed thoroughly with anhydrous ether and dried in a vacuum over paraffin. There was thus obtained 6.4 g. (60% of that calculated on the basis of glucose-anilide) of a slightly yellow powder; m. p., 129–132°; $[\alpha]_D^{23}$ = —47.6° for a 3% solution in methyl alcohol. Glucose-anilide browns at 140° and melts at 147°.[6] The specific rotation in methyl alcohol[5] is recorded to be —52.3°.

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[6] Sorokin, *Ber.*, **20**(R) 783 (1887).

The material was recrystallized four times by dissolving in absolute ethyl alcohol filtering while hot and precipitating cold with anhydrous ether. There was finally obtained 3.4 g. of a pure white material; m. p., 146°; $[\alpha]_D^{23}$ = —51.6° for a 3% solution in methyl alcohol.

Anal. Calcd. for $C_{12}H_{17}O_5N$: N, 5.49. Found: 5.26.

That glucose-anilide is responsible for the color in acid solutions of glucose and aniline is indicated by the fact that this substance in alcohol becomes colored and more rapidly so if acetic acid is present. Two solutions of a pure glucose-anilide were made up as in Table II below; the color of the solutions was noted and the rotation followed in order to determine the effect of acid.

The glucose-anilide was prepared in the usual manner;[6] it was recrystallized four times from methyl alcohol and washed with anhydrous ether. A 3% solution in methyl alcohol gave $[\alpha]_D^{22}$ = —52.22°.

TABLE II

ROTATION AND COLOR OF GLUCOSE-ANILIDE IN ETHYL ALCOHOL

Glucose-anilide, 2.125 g. Solvent, 95% ethyl alcohol to volume of 50 cc. Tube, 1dcm. Temp., 21°.

	No acid		Acetic acid, 0.5 g.	
Time	α	Color	α	Color
5 min.			−1.32	Colorless
30 min.	−0.72	Colorless	1.42	Colorless
1 day	1.47	Colorless	1.34	Slight yellow
4 days	1.37	Slight yellow	1.28	Decided yellow
6 days	1.35	Yellow	1.25	Orange
24 days	1.30	Decided yellow	?	Red-brown
47 days	?	Orange		

In the absence of acid, the solution of glucose-anilide gradually became more negative; according to Irvine and Gilmour[5] this is due to the conversion of the alpha to the beta isomer. The maximum value (—1.47°) was recorded in 24 hours; thereafter the slight rise in rotation was probably due to hydrolysis. With acid present, the maximum value (—1.42°) was noted in half an hour. This corroborates the findings of Irvine and Gilmour that the *d*-isomer is rapidly changed to the *l*- in presence of acid. Both solutions became colored but the acid solution much more rapidly than the other.

In 40 days the brown acid solution was diluted to 400 cc. with water and the colored precipitate removed. After drying in a vacuum, it weighed 0.26 g. and melted vaguely between 94° and 150°.

This simply indicates that from acid solutions of glucose-anilide a colored material can be obtained similar to that from an acid solution of glucose and aniline.

Finally, the effect of changing the concentration of aniline and of aniline and acetic acid was studied. In the case of aniline alone, the solutions were made up as given in Table III with acid present as in Table IV.

TABLE III

EFFECT OF INCREASE IN CONCENTRATION OF THE ANILINE ON THE PRODUCTION OF GLUCOSE-ANILIDE

Glucose, 1.5 g. Solvent, ethyl alcohol to volume of 50 cc. Tube, 1 dcm. Temp., 22°.

Aniline Time	4.65 g. 6-mol. proportions α	6.975 g. 9-mol. proportions α
5 min.	+1.73	+1.73
6 hours	+1.77	+1.78
1 day	+1.78	+1.73
6 days	−0.30	−0.39
12 days	−1.80	−1.94
20 days	−2.12	−2.23
21 days	−2.14	−2.25[a]
22 days	−2.17[a]	−2.25
23 days	−2.16	−2.23

[a] Maximum values observed.

Again at the start, using aniline and glucose, a slight increase in rotation is noted, presumably due to the formation of the alpha isomer. The effect is not so marked as that obtained by Irvine and Gilmour but they used a more concentrated sugar solution and a lower temperature (2–6°). After the first day the rotation gradually falls until in 21 and 22 days, respectively, the maximum values for the 6 and 9 molecular proportions of aniline was noted.

TABLE IV

EFFECT OF INCREASE IN CONCENTRATION OF ANILINE AND ACETIC ACID ON THE PRODUCTION OF GLUCOSE-ANILIDE

Glucose, 1.5 g. Alcohol to 50 cc. Tube, 1 dcm. Temp., 21°.

Aniline, mol. pro.	3	6	9
Acetic acid, mol. pro.	3	6	9
Time, hours	α	α	α
$^{1}/_{12}$	+1.52	+1.20	+0.93
1	−0.40	−1.57	−1.98
2	−1.28	−2.02	−2.10[a]
4	−1.80	−2.07[a]	−2.09
6	−1.92	−2.05	−2.07
24	−1.95[a]	−2.02	red
48	−1.90	red	
96	red		

[a] Maximum values observed.

With acid present, as before, the preliminary formation of the alpha isomer was not observed, the rotation rapidly falling to its maximum value. The effect of the acid in speeding up the reaction is very noticeable; for instance, using 6 moles of aniline alone, the maximum value was recorded in 22 days, while with 6 molecular proportions of acid and aniline the maximum value was noted in four hours. The effect of acid on the rate of reactions such as ester formation is, of course, well known. The interest in this particular case lies in the fact that reactions between amines, which are bases, and glucose can be speeded up by acids and at the same time the solution becomes neither basic nor strongly acid. It becomes, in effect, a reaction of glucose near the neutral point. This will be dealt with later.

A rough idea of the percentage formation of glucose-anilide under different conditions can be calculated by using the maximum rotations observed and the specific rotation of glucose-anilide in alcohol. The latter value was determined as follows in presence of excess aniline to prevent hydrolysis.

1.4998 g. of a pure glucose-anilide was dissolved in ethyl alcohol, 3.87 g. of aniline added and the volume made up to 50 cc. In 35 minutes, the rotation in a 1dcm. tube was +0.01°. This positive value is due to the presence of the alpha isomer, but as this is gradually converted into the beta, the rotation slowly falls, so that in 11 days a maximum value of —1.67° was recorded. Thereafter the rotation very slowly became less negative due to hydrolysis, but even in 30 days it was —1.62°. The maximum rotation gives $[\alpha]_D^{21}$ —53.01° for glucose-anilide in the presence of excess of aniline.

The calculated amount of glucose-anilide that could be formed from 1.5 g. of glucose and the requisite amount of aniline is 2.125 g., and this should give α, —2.25. The values obtained are given in Table V.

TABLE V

SPECIFIC ROTATIONS OF GLUCOSE-ANILIDE

Aniline, molecular proportions	Acid, molecular proportions	Max. $[\alpha]_D$ obs.	Glucose-anilide %
1	0	−1.28	56.9
6	0	−2.17	96.4
9	0	−2.25	100
1	1	−1.25	55.6
3	3	−1.95	86.7
6	6	−2.07	92.0
9	9	−2.10	93.3

Summary

Aromatic amines, such as aniline, act on glucose in the presence of acetic acid to produce a brown material that is insoluble in water. Likewise, glucose-anilide in the presence of acetic acid becomes colored to yield a similar product. As glucose-anilide is formed in acid solutions of glucose and aniline and as the formation of this compound is catalyzed by acid, it is believed that the initial step in the production of the colored material is the formation of glucose-anilide.

Glucose-anilide in ethyl alcohol containing an excess of aniline to suppress hydrolysis gave $[\alpha]_D^{21}$ = —53.01°.

The effect of using an excess of the reactants, aniline and acetic acid, on the formation of glucose-anilide is noted; unless an excess is used, the reaction is far from complete.

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